Description

Electrochemical transducer array and use thereof

The invention relates to an electrochemical transducer array, and to specific uses of a transducer array such as this.

Electrochemical transducers are generally subdivided into the three groups of potentiometric, conductometric and amperometric. In the case of potentiometric transducers, the potential is measured with respect to a reference electrode. Ion-selective sensors operate on this basis, and the electrode is in this case coated with an ion-selective membrane. The potential on the electrode is then a measure of the concentration of the corresponding ions. A potentiometric pCO_2 sensor can thus also be produced by means of a gas-permeable membrane.

In the case of amperometric transducers, in contrast, a voltage difference is produced between two electrodes, in the case of which the substance to be detected is converted. The currents which flow during the reduction or oxidation process result in the measurement signal. These are widely used as oxygen sensors or biochemical sensors. In the case of a Clark-analogous oxygen sensor, a gas-permeable membrane is applied to the amperometric sensor. In the case of biochemical sensors, molecular identification systems, such as haptens, antigens or antibodies are placed on or in the vicinity of the electrodes. The target molecule binds thereto and is provided either directly or via intermediate steps with an enzyme label. If the corresponding enzyme substrate is now added, the enzyme releases a substance which can be detected. This is done either optically or electrochemically. This is the so-called ELISA test (Enzyme Linked Immuno Sorbent Assay). DNA analysis methods can also be carried out in a similar way.

The transducers which are used for electrochemical detection must include electrodes with which electrical contact is made individually. During use of potentiometric transducers, the resultant equilibrium potential with respect to a reference electrode must be able to be measured. In the case of amperometric and conductometric transducers, it must be possible to potentiostat the electrodes, and it must be possible to detect the current flow through the electrodes individually.

One example of planar ion-selective sensors is described in E. Jacobs et al, "Analytical Evaluation of i-STAT Portable Clinical Analyzer and Use by Nonlaboratory Health-Care Professionals", Clinical Chemistry, 39, 1069 et seq. (1993). This is a silicon substrate with thin-film electrodes and ion-selective membranes. The sensor electrodes and contacts are in this case located on the same side of the silicon substrate. In order thus to separate the contact surfaces and the flow cell for the analyte, the substrate must be considerably larger than the area which is actually required by the sensors.

Various biochips are likewise manufactured using silicon technology, and are described R. Thewes et al, "Sensor Arrays for Fully Electronic DNA Detection on CMOS", ISSCC Digest of Tech. Papers, 2002, 350 et seq. This has the advantage of the integration of CMOS circuit technology, signal processing (multiplexing) and analog/digital conversion in the sensor platform itself. A large number of sensors can thus be provided in a very small area. One disadvantage relates to the costs for production of a chip such as this and the complex handling (contact-making). The costs per individual sensor are thus high for so-called low-density arrays with fewer than 100 sensors per square centimeter.

Theoretically, it is possible to use polymer mounts with electrodes fitted to them, as an alternative. These can be

vapor-deposited or printed on. This method makes it possible to produce individual sensors, for example glucose sensors, at low cost [WO2002/02796-A2]. However, it is not very suitable for arrays since the conductor track structures are coarse, so that the number of electrical contacts is greatly restricted.

Printed circuit board technology is used in the already known eSensor™ from the Motorola Company in order to produce a "low-density" DNA detection system. In this case, both the sensor surfaces and the conductor tracks and contacts are formed on the metallization layer. The product is a rigid printed circuit board with sensors and contacts on the same side. Rear-face contacts can be provided by through-plating. This technique can, however, be implemented only at high cost for large-scale manufacture.

Furthermore, by way of example, so-called microelectrode arrays are known from EP 0 504 196 B1 and DE 197 17 809 U1, in which the sensor cavities have as small an area as possible. DE 199 16 921 A1 discloses a method for production of arrays which are arranged in pairs and are composed of microelectrodes, in which the mount is either silicon or plastic. The aim in this case is to be able to drive the individual electrodes separately. DNA analysis is quoted in particular as an application.

Finally, WO 2004/001404 A1 discloses an array of microelectrodes in which the structure can be varied. The array mounts are in this case glass and/or Captan films, with a single reference-ground electrode being used. Finally, DE 199 29 264 A1 discloses a universal transducer for chemosensors and biosensors, in which a multilayer system is provided with isolating layers and electrode layers, which are used as working, reference-ground and counterelectrodes.

The large number of known transducer arrays therefore place particular emphasis on specific microelectrodes, with contact always being made from above.

Against the background of the overall prior art as cited above, one object of the invention is to provide a suitable transducer array which is simple to handle and can be produced at low cost. A further object is to propose advantageous uses of the transducer array.

According to the invention, the object is achieved by the features of patent claim 1. Developments are specified in the dependent claims. Preferred uses of the transducer array according to the invention are the subject matter of claims 26 and 27.

In the transducer array according to the invention, at least one flexible, planar metal substrate is provided, on which at least one flexible isolator is arranged with a permanent connection between the metal surface and the isolator surface. In this case, both the self-supporting metal substrate and the isolator are structured in such a manner that metal surfaces are formed which are electrically isolated from one another and provide the sensor surfaces, in which case the structured metal areas of the self-supporting metal substrate can be contacted from the side facing away from the sensor surface or the side opposite the sensor. This results in a simple measurement capability by means of needle contacts, particularly for decentralized measurement by means of smart cards.

One particularly advantageous feature of the invention is the good handling capability of the product. The product is a material composite which is only 100 µm to 200 µm thick and can occupy any desired area. The sensor array is thus highly flexible and, with an appropriate geometry, can be guided on

rollers. In the simplest case, the composite comprises a metal layer and an isolator layer. The front face of the

metal substrate is covered by the isolator, with only small metal surfaces remaining, which represent the sensors. Generally, the sensors have to be resistant to aqueous electrolytes and also have to have catalytic activity for the conversion of the chemical substance to be detected. In order to achieve this, they can be coated with noble metals such as platinum, gold or silver. Depending on the requirements of the circuit technology, some areas can be in the form of reference electrodes or counterelectrodes. In particular, it is possible to use a sensor surface coated with silver and chlorided as a reference electrode.

The metal layer can advantageously be used on both sides. The sensors are located, as described, on the front face. The rear face is used to make contact with the sensors. In this case, the metal layer is structured such that each sensor is electrically isolated from the others. The rear-face metal surface which this results in and which corresponds to a single sensor on the front face is considerably larger than the sensor surface. Contact can thus be made at a point which is not located directly underneath a sensor surface and is reinforced by the isolator. Since the metal substrate is self-supporting, the rear-face contact may, however, also be made directly underneath the sensor surface in order in this way to allow a particularly space-saving embodiment. One proposed way to make contact is to use needle cards, which are also used in the application examples.

In the case of a very large sensor array in the form of a strip, it is possible not to make contact with all of the sensors at the same time, but to move in the form of a magazine through the measurement apparatus. The needles would automatically make contact with the sensor surfaces at that time, with the array element being available as an "endless array" for the measurements. This procedure is particularly important for use for automatic monitoring of processes.

Titer plates play an important role in analysis (for example HTS: High Throughput Screening). These contain 96 (8*12), 384 (16*24) or 1536 (32*48) small plastic reaction pots with grid sizes of 9 mm, 4.5 mm and 2.25 mm, respectively. In some cases, optical detection processes can be carried out directly using titer plates such as these. For this purpose, the titer plates have, for example, planar, optically transparent bases. The transducer arrays according to the invention can in this case advantageously be used for electrochemical detection. For this purpose, they are matched to the external dimensions of the titer plates and to the grid side of the small reaction pots. They form the base of the titer plates, so that each small reaction pot has at least one associated electrode. Since contact can be made with the rear face of the transducer arrays according to the invention, contact can be made at the same time with all of the electrodes on the titer plate, and they can thus be read at the same time.

A further advantage of the transducer array according to the invention, particularly in comparison to silicon-chip technology, is the structure of the array surface. This is not flat. Instead of this, each sensor is located in a depression, which is predetermined by the thickness of the isolator used. These cavities are particularly suitable for accommodation of coatings. They may contain the traps that have been mentioned for DNA analysis, antibodies or selective membranes.

In one specific embodiment, the cavity may even represent a closed electrochemical system. At least one second electrode is required per cavity for this purpose. This can be formed either by division of the sensor surface or by the introduction of a further electrode, which is placed over the cavity as a cover. In this case, this cover is not a fixed component of the sensor array, since the analyte must first be introduced into the cavity. By way of example, it may likewise be joined to the

sensor array as a strip. The advantage of a closed arrangement such

as this is that the substance to be detected is enclosed in the cavity. It can neither diffuse away, thus attenuating the signal, nor can it reach another sensor where it would result in an incorrect signal.

Further details and advantages of the invention will become evident from the following description of the figures of exemplary embodiments, on the basis of the drawing in conjunction with the patent claims.

In the figures, in each case illustrated in a schematic simplified form:

Figure 1 and Figure show the front face and rear face of a transducer array,
Figure 3 shows a section illustration of a transducer array as shown in Figures 1/2,
Figure 4 shows a plan view of a two-dimensional array,
Figure 5 shows a section illustration, as a partial detail of the transducer array shown in Figure 4 with the associated contact,
Figures 6 to 14 show section illustrations of various variants of a transducer array as shown in Figures 1/2,
Figure 15 shows a measurement apparatus using a transducer array as shown in Figures 3 to 14,
Figure 16 shows the results of use of a transducer array as an ion-selective sensor, and
Figure 17 shows results of the use of a transducer array corresponding to one of Figures 1 to 14 as a DNA sensor.

Figures 1 and 2 show the front and rear faces of a sensor array comprising a metal substrate 1 and an isolator layer 2. By way of example, circular depressions 3_i, which are referred to as cavities, are illustrated on the front face. The cavities 3_i are produced by the structuring of the isolator 2. The surface

of the metal substrate at the bottom of the depressions 3_i is exposed.

The illustration of the rear face uses oblique lines to show the subdivision of the metal substrate 1 into parts 10_i which are isolated from one another. Each metal island 10_i corresponds to the cavity 3_i of an isolator cutout on the front face. The possible contact points for a so-called needle card for selectively making electrical contact with the metal surfaces are indicated by dots on the rear face.

Figure 3 shows a side view of a sensor array, in the form of a section through one row of electrodes or sensors. The separating lines in the metal substrate 1 are illustrated as singular measurement electrodes 10_i with a measurement area 12_i and an opposite face as a contact-making surface 11_i. The isolator 2 is located above this, is composed of individual elements 20_i, holds the self-supporting metal surfaces together, and isolates them from one another.

Figure 4 shows a plan view of a two-dimensional mxn sensor array, in which the cavities 3_i and the measurement surfaces 12_i are located close to one another. The adjacent cavities 3_i and 3_{i+1} with measurement surfaces are indicated in the array, in which case the aim is to be able to make contact with the array on the side 11_i facing away from or opposite the sensor surface 12_i. While one sensor is directly adjacent to the other sensors in the area of the mxn array, a side metal area remains free on the rear face of the outer sensor row, for making contact with.

Figure 5 shows a detail of the sensor array from Figure 4 with electrodes fitted from the lower face of the metal substrate 1 in order to tap off measurement signals. The measurement technique with the associated measurement apparatus and the electrode arrangement that is advantageously used in this case will be described in detail further below with reference to Figure 10.

In the case of the first sensor surface, one contact 4a is fitted to the metal surface 11_i, which is exposed on both sides, centrally, directly

opposite the sensor surface. In contrast, in the case of the second sensor surface, contacts 4b may be fitted to the metal surface, which is exposed on one side, laterally offset with respect to the sensor surfaces, since there is sufficient remaining space here.

Figure 6 shows a sensor array with two electrodes per cavity. For this purpose, the metal substrate is split at this point. The resultant gap is closed by an additional isolator layer 40_i from the lower face. In this case, contact surfaces remain free and define measurement electrodes. A working electrode WE and a counterelectrode CE are introduced alternately.

Figure 7 shows that a plurality of cavities are wetted by the same electrolyte. The metal surface of one cavity can then be polarized in the opposite direction to the metal surface of another cavity.

Figure 8 shows that one of the open metal surfaces is covered on the front face by a thin silver/silver chloride layer. This layer 40_1 can be connected to a potentiostat, together with two further wetted metal surfaces, in a three-electrode arrangement as a working electrode (WE), a counterelectrode (CE) and a reference electrode (Ref).

Figure 9 shows the use of an external reference electrode 15, which is immersed in the common electrolyte which also wets at least two metal surfaces. Together, they can be connected to a potentiostat in a three-electrode arrangement.

Figure 10 shows an external reference electrode which is immersed in the same electrolyte as that which also wets a plurality of cavities, each having two electrodes. The two electrodes together with the reference electrode in each case form a three-electrode arrangement.

Figure 11 shows that the electrolyte areas in each cavity can be electrically isolated from the other electrolyte areas.

Figure 12 shows that an electrical conductor which is located above the cavities can be used as a common counterelectrode CE for all of the cavities. A voltage is in each case applied between the metal surface in the cavity and the common counterelectrode.

Figure 13 shows that, in the case of a sensor array with two electrodes per cavity 3_i and 3i, one of the two electrodes is coated with silver/silver chloride (Ag/AgCl). This coated electrode is connected as a reference electrode to a potentiostat, together with the second electrode in the cavity as the working electrode, and the covering counterelectrode, in a three-electrode arrangement.

Figure 14 shows that an electrode which covers the measurement arrangement is coated with silver/silver chloride on the electrolyte side. The sensor array has two electrodes per cavity. A three-electrode arrangement can thus be produced with these two electrodes as the working electrode WE and the counterelectrode CE, and with the covering electrode as the reference electrode.

Figure 15 illustrates the measurement apparatus in detail. In this case, use can be made of the method of "pulsed" redox cycling, which is described in detail in a parallel application from the same applicant, with the same application priority, and entitled "Method for measurement of the concentration or concentrate change of a redox-active substance, and an associated apparatus".

Apart from being formed by a transducer array 100, various variants of which have been described on the basis of the

Figures 3 to 14, the measurement layout is essentially provided by a suitable potentiostat 5 in combination with a pulse

generator 6, which optionally produces square-wave, triangular-waveform or sinusoidal pulses. The potentiostat 5 is designed in such a manner that suitable potentials are produced, by means of two operational amplifiers 7 and 7', one of which is connected to a "ground" potential, and to one defined measurement resistance. In this case, the pulse duration, the repetition rate and the magnitude of the potential can be predetermined. In particular, the pulse durations of the measurement phases and the relaxation phases can be adjusted separately, and may be of different duration. The potentials may also be of different magnitudes.

The transducer array 100 is associated with the individual electrodes which, by definition, provide a reference electrode RE, a counterelectrode CE and at least one working electrode WE. These electrodes are connected to the potentiostat 5 as a three-electrode arrangement. The signal from the potentiostat 5 is connected to a signal processing unit, which is not illustrated in detail in Figure 9 but by means of which an evaluation process is carried out, taking into account the above statements relating to the measurement method and accuracy. In general, this results in $U_{out} \sim I$ for evaluation of the signal profile illustrated in Figure 15.

In one specific development, a transducer array corresponding to one of the examples described above is used as an ion-selective sensor: a sensor array comprising a metal layer and an isolator layer is used for this exemplary application. The diameter of the cavities is 0.8 mm, the depth is 90 µm, and the distance between two adjacent electrodes is 1 mm. The electrode surfaces are covered with a 2.3 µm thick gold layer. Overall, the array comprises four electrodes, one of which is in the form of a silver chloride reference electrode. The other three electrodes have been coated with an ion-selective membrane. An ammonium-selective membrane is quoted as one example here.

Corresponding to the recommendation of Fluka, the membrane composition was:

- 1.00% by weight Ammonium Ionophore I (Fluka 09877)
- 33.00% by weight Poly(vinyl chloride) high molecular weight (Fluka 81392)
- 66.00% by weight Dibutyl sebacate (Fluka 84838)

A total of 100 mg of the reagents was dissolved in 550 µl of a mixture of cyclohexan and THF, in the ratio 8:2. In each case 35 nl, 45 nl and 60 nl of this solution were spotted into the three sensor cavities, thus resulting in three membranes of different thickness. These were dried for several hours in air.

The sensor array was inserted into a 100 µm deep through-flow channel, and solutions of different NH_4NO_3 concentrations were then pumped over it. The solutions also included 100 mM of Tris(hydroxymethyl)aminomethane/hydrochloric acid for buffering at pH 8. The potential difference between the membrane-coated electrodes and the reference-ground electrode was then measured using a high-impedance ohmmeter. The following figure shows the potential change on the sensor as a function of the NH_4^+ concentration for the three membrane thicknesses.

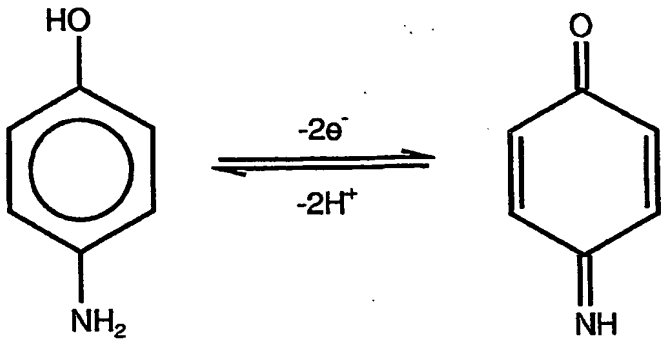
Figure 16 shows the relationship between the potential and the acid concentration. The concentration of NH_4NO_3 is plotted on the abscissa in mol/l, and the electrochemical potential φ with respect to an Ag/AgCl electrode is plotted on the ordinate. The graphs 161 to 164 show characteristics for different membranes.

The gradients of the regression lines are 54 mV, 52 mV and 48 mV from the thinnest to the thickest membrane. These values are somewhat less than the theoretical value of 59 mV at room temperature.

In another development, a transducer array corresponding to one of the examples described with reference to Figures 3 to 14 is used as a DNA sensor:

The sensor array that is used corresponds to the arrangement that has already been described in the previous example, with four electrode surfaces being used. One of the electrode surfaces is in the form of a reference electrode Ref, another is used as a counterelectrode CE, and the two other electrode surfaces are used as measurement electrodes or so-called working electrodes WE. On one of the working electrodes, a synthetic oligonucleotide sequence of length 25 is anchored on the gold surface by means of a terminal thiol group. The second measurement electrode remains free.

Both surfaces were incubated with a solution of 1 mg of bovine serum albumin per milliliter for 15 minutes, and the sensor array was then inserted into a 100 µm deep through-flow channel. First of all, 10 µl of a 10 µM biotinilated target sequence are pumped over the electrodes within about 5 minutes. After a washing step, a solution of streptavidin-labeled alkaline phosphatase is then passed over it. The washing is carried out using a buffer solution of 100 mM tris(hydroxymethyl)aminomethane titrated to pH 8 with hydrochloric acid, 130 mM NaCl. After washing again, a 2 mM solution of the enzyme substrate paraaminophenyl phosphate (pAPP) in the buffer solution is pumped over the sensor array. In the presence of the enzyme alkaline phosphatase, the enzyme substrate pAPP is converted to paraaminophenyl (pAP). The pAP is oxidized, with an appropriate potential on the electrode, to form quinonimine. This process can also be reversed, with the quinonimine being reduced to pAP again. In this case:



paraminophenol **quinonimine**

The reference electrode, counterelectrode and in each case one of the two measurement electrodes are located in a three-electrode arrangement connected to a potentiostat. Owing to the large electrode areas, a potentiostatic measurement method would lead to major depletion of the pAP. A suitable pulsed process is therefore used.

At the start of the measurement, the positive sample, that is to say the electrode with the trap sequence, is connected. The solution with the enzyme substrate first of all flows over the negative sample, then over the positive sample. The flowing movement flushes pAP formed from the enzyme away from the electrodes, so that the current is constant and low when the pump is switched on. If the pump is now stopped, the pAP concentration rises with time because of the enzyme activity. This is evident in the measurement by a major rise in the current signal at 20 nA/s. If the pump is switched on again, then the signal falls to the original value again. This process can be repeated as often as desired.

Figure 17 shows the profile of the measurement current with the pump "on"/"stop" at the sensor with a positive and negative sample. The graph shows the time t in s on the abscissa, and the current I in nA on the ordinate. The graph 171 shows the measurement current in the profile during an experimental investigation.

The negative sample was switched over at t = 400 s. Here, the current first of all falls when the pump is stopped, then remains constant for a short time, and then rises slowly. This rise is caused by the diffusion of pAP from the positive sample to the negative sample. When the pump is on, a peak current is added, since the electrolyte first of all flows from the positive sample to the negative sample, and thus transports an increased pAP concentration to the adjacent electrode. Overall, this results in very good discrimination of the positive and negative sample.